John von Neumann Institute for Computing

Single Molecule FRET Study of the Conformational Energy Landscape of a Transfer RNA

A. Yu. Kobitski, M. Hengesbach, M. Helm, G. U. Nienhaus

published in

From Computational Biophysics to Systems Biology (CBSB08),
Proceedings of the NIC Workshop 2008,
Ulrich H. E. Hansmann, Jan H. Meinke, Sandipan Mohanty,
Walter Nadler, Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **40**, ISBN 978-3-9810843-6-8, pp. 253-256, 2008.

http://www.fz-juelich.de/nic-series/volume40

Single Molecule FRET Study of the Conformational Energy Landscape of a Transfer RNA

Andrei Yu. Kobitski[1], **Martin Hengesbach**[2], **Mark Helm**[2], **and G. Ulrich Nienhaus**[1]

[1] Institute of Biophysics, University of Ulm, 89069 Ulm, Germany
E-mail: andrei.kobitski@uni-ulm.de, uli@uiuc.edu

[2] Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, 69120 Heidelberg, Germany
E-mail: martin.hengesbach@web.de, mark.helm@urz.uni-heidelberg.de

Local, post-transcriptional modifications can have pronounced effects on the global energy landscape of RNA molecules. We have studied the equilibrium between discrete conformational states in human mitochondrial lysine transfer RNA (mt $tRNA^{Lys}$) by using single-molecule Förster (or fluorescence) resonance energy transfer (smFRET) spectroscopy. From histograms of the FRET efficiency, an unfolded structure (***U***), a non-functional, extended hairpin structure (***E***) and the functional cloverleaf form (***C***) of human mt $tRNA^{Lys}$ can be distinguished. The equilibria between the ***U***, ***E***, and ***C*** states were characterized as a function of Mg^{2+} concentration for two RNA constructs that only differ by a single methyl group modification of a nucleotide base. A thermodynamic model was developed which is based on the separation of conformational changes and binding of divalent cations. Based on this model, the impact of a single methyl group modification on the energy landscape of $tRNA^{Lys}$ was assessed.

1 Introduction

Proteins and ribonucleic acids (RNAs) are linear polymers that can fold into compact three-dimensional structures, in which they are able to perform specific roles in biological processes within living cells or organisms. Finding the correctly folded structure is an extraordinarily complex process that has yet to be solved by *in-silico* modeling approaches, although significant progress has been made over the years[1]. The key problem for computation is the vast conformational space of even moderately sized biopolymers[2]. The conformational energy landscape has provided a conceptual framework by which to describe both protein folding and function[3]. Protein folding is visualized by a transition of the molecular ensemble on the energy landscape via many parallel trajectories and local minima en route to the folded-state ensemble. Folding of proteins and RNA is overall governed by the same principles; yet there are differences arising from the nature of the interactions introduced by the monomeric units. A distinct difference, however, is the hierarchical nature of RNA folding that originates from the pronounced base pairing leading to the formation of relatively stable secondary structures[4].

Frequently, post-transcriptional chemical modifications of ribonucleotides are observed which only slightly change the energy landscape to selectively stabilize the native conformation[5]. Here we present an exception, human mitochondrial (mt) lysine transfer RNA ($tRNA^{Lys}$), in which a single methylation on adenosine 9 (m^1A9) in its structural core was seen to cause a marked shift of the thermodynamic equilibrium toward the functional form of the RNA molecule[5,6]. We have studied this biologically important modification by using single-molecule Förster (or fluorescence) resonance energy transfer

(smFRET), a technique that allows conformational changes of biomolecules to be visualized under equilibrium conditions in real time[7]. It relies on the ability of a fluorescent dye (donor) to transfer its energy non-radiatively to another fluorescent dye (acceptor) that typically absorbs at longer wavelengths. By attaching such a FRET pair of dyes specifically to the structure of interest, the strong distance dependence (R^{-6}) of the effect enables distance changes down to 1 Å to be measured. Such quantitative experimental data are expected to be most useful in the development of both theories and computational modelling approaches.

2 FRET Measurements

Two suggested secondary structures of tRNA^{Lys}, the non-functional extended hairpin (***E***) and the functional cloverleaf-based L-shape (***C***) conformations, are presented in Fig. 1a. To observe the conformational changes between ***E*** and ***C***, induced by the methylation of adenosine 9 (A9), two FRET constructs with the unmodified (Kwt) and the modified (Km^{1}A) sequences were prepared[6]. The presence of multiple conformations in the FRET-labeled RNA constructs was investigated by smFRET experiments on freely diffusing and surface-immobilized molecules using a confocal microscope. In these experiments, the efficiency of FRET, $E = \frac{I_A}{I_A+\gamma I_D}$, is calculated ratiometrically from the donor (I_D) and (I_A) acceptor fluorescence photon counts, and the parameter γ accounts for differences in the donor and acceptor quantum yields and the detection efficiencies. RNA is a polyanion, carrying one unit of charge on each nucleotide, and the Coulomb repulsion must be screened by counterions, which may bind in specific locations or just form a diffuse cloud, to stabilize the functionally competent states. Variation of the counterion concentration is a useful experimental control parameter to shift equilibria between different structures in the energy landscape. We have measured histograms of the FRET efficiency at 16 different Mg^{2+} concentrations, which can be described by superpositions of three FRET efficiency distributions peaking at low, intermediate and high FRET values. Based on the proposed structural model for tRNA^{Lys} and the crystal structures of tRNA^{Phe}, we have assigned these subpopulations to the ***U*** (for "unfolded"), ***E*** (for "extended hairpin"), and ***C*** (for "cloverleaf-based L-shape") states. Switching events between the ***E*** and ***C*** states, with

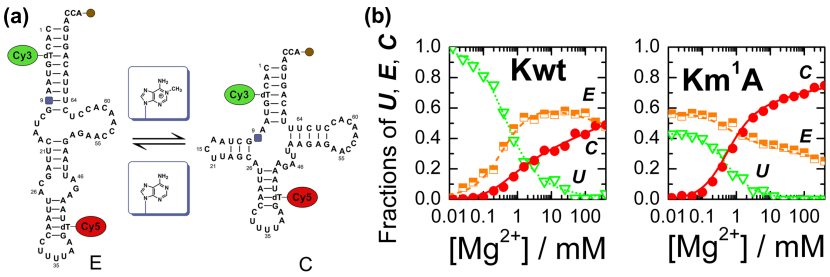

Figure 1. Conformational changes of human mt tRNA^{Lys} affected by methylation of adenosine 9 (square). (a) Secondary structures of the proposed extended hairpin (***E***) and the cloverleaf-based L-shape (***C***) conformations. (b) Mg^{2+} dependence of the fractional populations in the unfolded (***U***), ***E***, and ***C*** states of Kwt and Km^{1}A tRNA^{Lys} constructs. Results from fitting the data with the thermodynamic model are given as lines.

rare brief sojourns to ***U*** states, were observed in FRET trajectories of individual molecules immobilized on a glass surface. At 10-mM Mg^{2+} concentration, the kinetics of $tRNA^{Lys}$ was described with a simple model involving two conformations, ***E*** and ***C***, interconverting on the 100-ms timescale[6]. To quantify the change of the ***U***, ***E***, and ***C*** conformations with Mg^{2+} concentration, we performed a global fit of the 16 FRET histograms for each of the constructs; in Fig. 1b, the fractional populations of the three states of Kwt and Km^1A are plotted. For both constructs, a pronounced drop of the ***U*** state population is observed at $\sim$0.5 mM Mg^{2+}. It is accompanied by an increase of the ***C*** state population with increasing Mg^{2+} concentration. The ***E*** state is much more populated at low concentrations in Kwt than in Km^1A. At high Mg^{2+} concentration ($\sim$100 mM), the ***E*** and ***C*** state populations decrease and increase with Mg^{2+}, respectively.

3 Thermodynamic Model

Ion-induced stabilization of RNA can be modeled by decomposing the reaction into RNA folding and ion binding, as proposed by Misra and Draper[8]. Based on this approach, we have developed the six-state thermodynamic model depicted in Fig. 2a. In this model, there are free energy differences between the Mg-free U_0, E_0, and C_0 conformations, whereas the strength of Mg^{2+} ion binding to the U_{Mg}, E_{Mg}, and C_{Mg} conformations governs the equilibrium between the Mg-free and Mg-bound populations of the corresponding states. Mathematical details of the thermodynamic model can be found in a recent publication[6]. The lines in Fig. 1b represent the fit results of the fractional populations governed by the equilibrium coefficients. The free energies of the Mg-free and Mg-bound (at 1 M) states are depicted in Fig. 2b. The compaction of the conformations going from U_0 to E_0 and from E_0 to C_0 increases the free energies of the Kwt construct. For the Km^1A construct, the methylated A9 introduces a positive charge, which influences base pairing[9] and stabilizes the E_0 state significantly ($\sim$10 kJ/mol). However, the stabilizing effect is drastically reduced for the E_{Mg} of Km^1A, possibly because of the competition between Mg^{2+} binding to the ***E*** state and favorable hydrogen bonding of m^1A9 in the base pair m^1A9-U64.

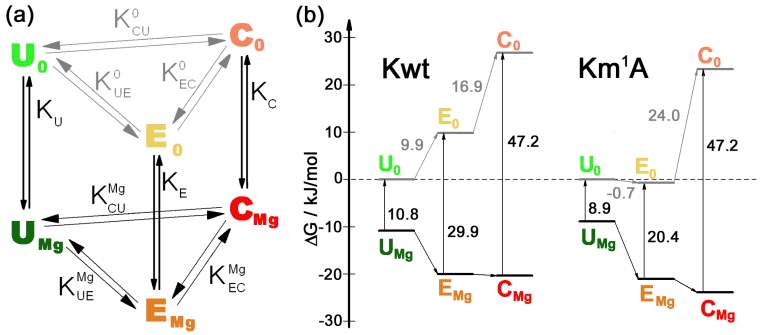

Figure 2. Mg^{2+}-induced $tRNA^{Lys}$ folding reaction. (a) Thermodynamic scheme describing the equilibria between the Mg-free and Mg-bound forms of the ***U***, ***E***, and ***C*** states. (b) Free energy diagram of the populations U_0, E_0, and C_0 of Mg-free, and U_{Mg}, E_{Mg}, and C_{Mg} (at 1-M Mg^{2+}) of Mg-bound Kwt and Km^1A $tRNA^{Lys}$.

In the cloverleaf conformation, A9 is likely exposed to the solvent and, thus, the stabilizing effect is smaller ($\sim$3 kJ/mol). Nevertheless, binding of Mg^{2+} ions remains practically unaffected, making this functional conformation thermodynamically preferable at physiologically relevant ion concentration.

4 Concluding Remarks

Here we have shown that FRET experiments performed at the single molecule level provide details about structural and dynamic aspects of RNA molecules. Equilibria and rate coefficients of conformational transitions can be studied by selectively changing environmental parameters, as was shown in here by variation of the counterion concentration. Quantitative information extracted from such measurement will be most interesting for comparison with computational approaches aimed at simulating these transitions in the complex RNA energy landscape.

References

1. W. F. van Gunsteren and J. Dolinc, *Biomolecular simulation: historical picture and future perspectives*, Biochem. Soc. Trans. **36**, 11-15, 2008.
2. A. V. Finkelstein and O. B. Ptitsyn, *Protein Physics*, Academic Press, San Diego, 2002.
3. J. N. Onuchic, Z. Luthey-Schulten, and P. G. Wolynes, *Theory of protein folding: the energy landscape perspective*, Annu. Rev. Phys. Chem. **48**, 545-600, 1997; K. A. Dill and H. S. Chan, *From Levinthal to pathways to funnels*, Nature Struct. Biol. **4**, 10-19, 1997; G. U. Nienhaus and R. D. Young, *Protein dynamics*, in: Encyclopedia of Applied Physics, G. L. Trigg (Ed.), VCH Publishers, New York, Vol. **15**, pp. 163-184, 1996.
4. P. Brion and E. Westhof, *Hierarchy and dynamics of RNA folding*, Annu. Rev. Biophys. Biomol. Struct. **26**, 113-137, 1997.
5. M. Helm, *Post-transcriptional nucleotide modification and alternative folding of RNA*, Nucleic Acids Res. **34**, 721-733, 2006.
6. F. Voigts-Hoffmann, M. Hengesbach, A. Yu. Kobitski, A. van Aerschot, P. Herdewijn, G. U. Nienhaus, and M. Helm, *A methyl group controls conformational equilibrium in human tRNA^{Lys}*, J. Amer. Chem. Soc. **129**, 13382-13383, 2007; A. Y. Kobitski, M. Hengesbach, M. Helm, and G. U. Nienhaus, *Sculpting an RNA conformational energy landscape by a methyl group modification - a single-molecule FRET study*, Angew. Chem. Int. Ed. **47**, 4326–4330, 2008.
7. G. Bokinsky and X. Zhuang, *Single-molecule RNA folding*, Acc. Chem. Res. **38**, 566-573, 2005; G. U. Nienhaus, *Exploring protein structure and dynamics under denaturing conditions by single-molecule FRET analysis*, Macromol. Biosci. **6**, 907-922, 2006.
8. V. K. Misra and D. E. Draper, *The linkage between magnesium binding and RNA folding*, J. Mol. Biol. **317**, 507-521, 2002.
9. P. E. Agris, H. Sierzputowska-Gracz, and C. Smith, *Transfer RNA contains sites of localized positive charge: carbon NMR studies of [13C]methyl-enriched Escherichia coli and yeast tRNA^{Phe}*, Biochemistry **25**, 5126-5131, 1986.